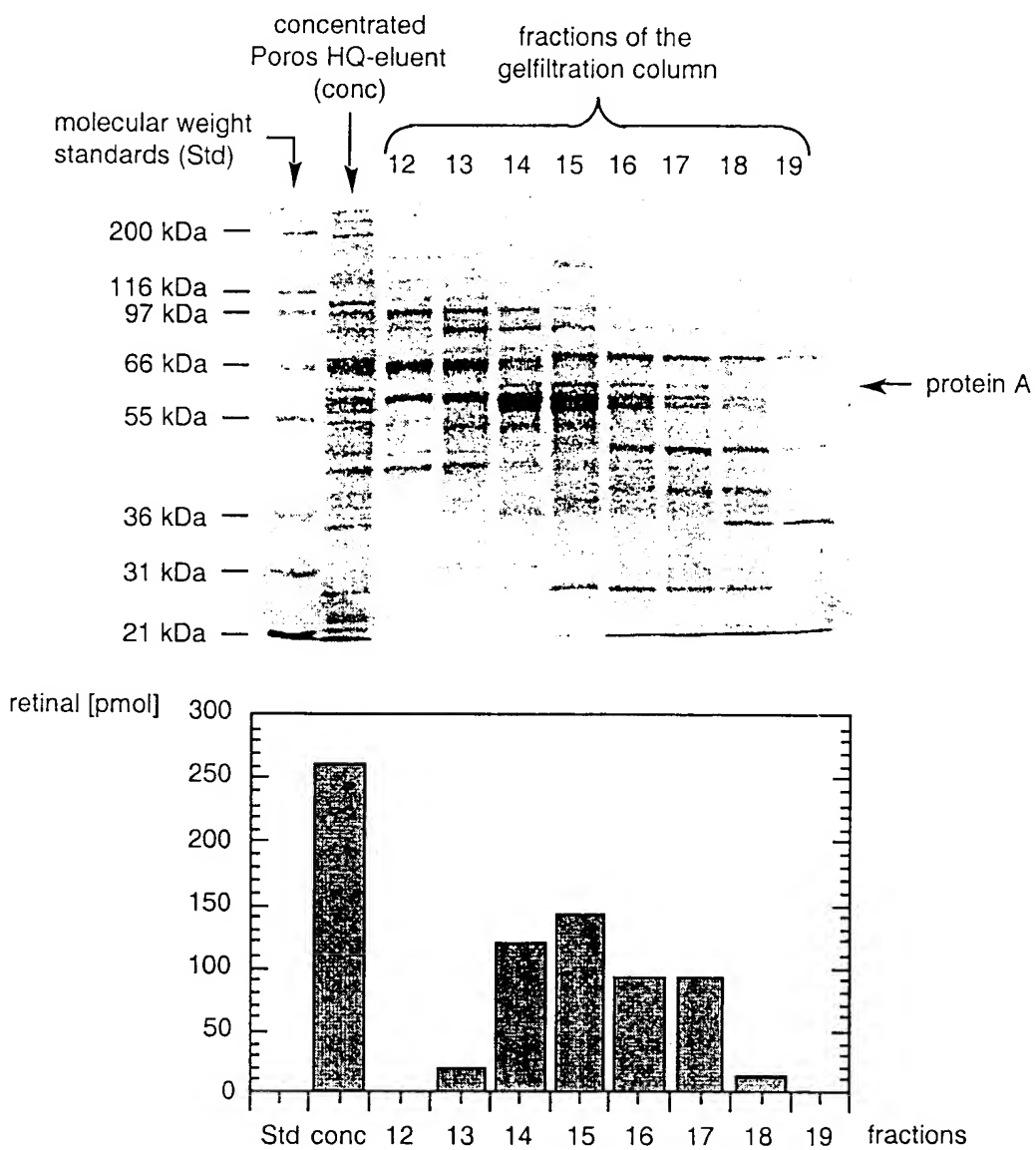


Figure 1



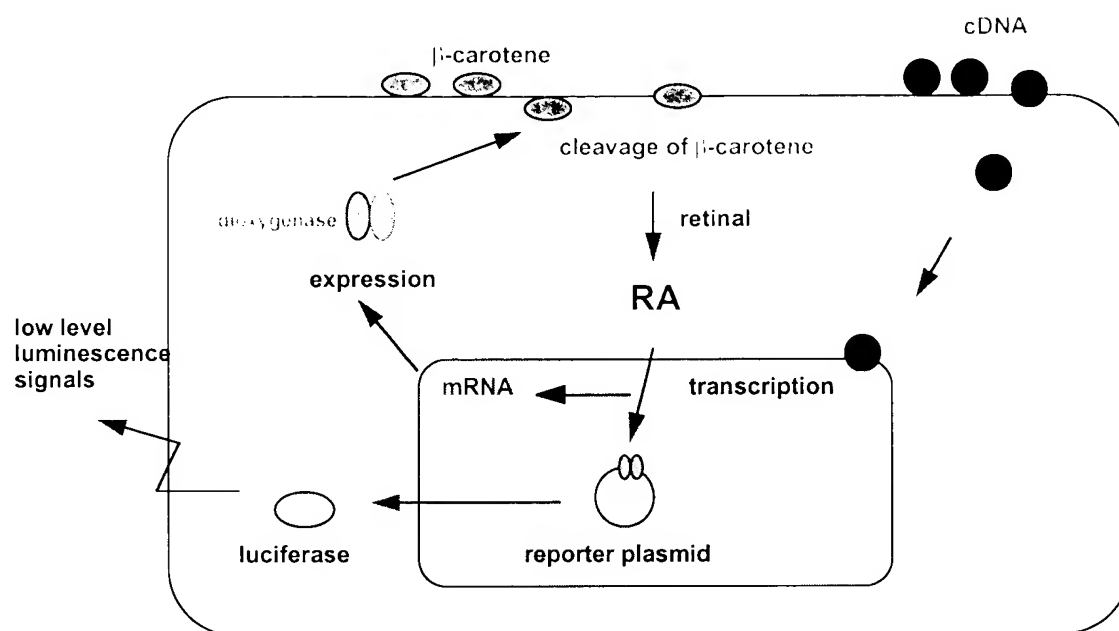


Figure 2

1 CGGATCCACT AGTAACGGCC GCCAGTGTGG TGAATCCAT
CCTTCTATGT

51 AACAGGAAAG AGCTGTTCTT AGCCAGAGA GGAGGGCACC
GTACGCCTGC

101 AGGAGCAGCT GGGTAGAGGA CACAGGAGAG CGATGGAGAC
AATATTTAAC

151 AGAAACAAAG AAGAGCATCC AGAGCCCATTA AAAGCTGAGG
TGCAAGGTCA

201 GTTGCCCACT TGGTTGCAAG GGGTACTTCT CCGAAATGGC
CCAGGGATGC

251 ACACAATAGG GGACACTAAA TACAACCACT GGTTTGATGG
CTTGGCTCTG

301 CTGCACAGCT TCACGTTTAA AAATGGTGAA GTTTACTACA
GAAGTAAGTA

351 CCTCCGAAGT GACACATACA ACTGCAATAT AGAAGCAAAC
CGAATCGTGG

401 TGTCTGAGTT TGAACCATG GCTTATCCGG ATCCATGCAA
AAACATATTT

451 GCCAAGGCAT TCTCATACTT ATCTCACACC ATTCTGAGT
TCACGGACAA

501 CTGCCTGATC AACATTATGA AAAGTGGGA TGATTATTAT
GCTACCAAGT

551 AGACTAACTT CATCAGAAAA ATTGATCCAC AGACTCTGGA
GACACTAGAT

601 AAGGTAGACT ACAGCAAATA TGTAGCTGTA AACTTGGCAA
CTTCTCACCC

651 ACACTATGAC AGTGCTGGAA ATATTCTCAA CATGGGTACT
TCAATTGTTG

701 ATAAAGGGAG AACAAAATAT GTTCTCTTTA AGATCCCTTC
CTCTGTACCA

751 GAAAAAGAAA AGAAGAAATC TTGTTTAAA CACCTGGAAG
TAGTATGCTC

801 CATCCCTTCT CGCTCCCTGC TCCAACCAAG CTAATACCAC
AGCTTTGGAA

851 TCACAGAAAA TTATATTGTG TTCATAGAGC AGCCATTTAA
ACTGGATATT

901 GTCAAACTGG CAACTGCCTA CATCCGAGGT GTGAACTGGG
CTTCCTGCCT

951 TTCCTTTCAT AAGGAGGATA AGACGTGGTT TCACTTTGTA
GACAGAAAGA

1001 CGAAAAAAGA AGTATCCACC AAGTTTACCA CTGATGCTTT
GGTGCTTTAT

1051 CACCACATAA ATGCTTACGA AGAAGATGGC CACGTTGTTT
TTGATATCGT

1101 TGCCTACAGA GACAATAGCT TGTACGATAT GTTTTACTTA
AAAAAACTGG

1151 ACAAAGACTT TGAAGTGAAC AACAAAGCTTA CCTCCATCCC
AACCTGCAAG

1201 CGCTTTGTTG TGCCTCTGCA GTATGACAAG GATGCAGAAG
TAGGTTCTAA

1251 TTTAGTCAAA CTTCCAACCT CCGCAACTGC TGTA AAAAGAA
AAAGATGGCA

1301 GCATCTATTG TCAACCTGAA ATATTATGTG AAGGGATAGA
ACTGCCTCGT

1351 GTCAACTATG ACTACAATGG CAAAAAATAC AAGTATGTCT
ATGCAACAGA

1401 AGTCCAGTGG AGCCCAAGTC CTACAAAGAT TGCAAAACTG
AATGTCCAAA

1451 CAAAGGAAGT ACTGCACTGG GGAGAAGACC ACTGCTGGCC
CTCAGAGCCC

1501 ATCTTTGTTC CCAGCCCCGA TGCAAGAGAA GAGGATGAAG
GTGTTGTTTT

1551 GACCTGTGTT GTGGTGTCTG AGCCAAATAA AGCACCCCTC
CTACTCATCT

1601 TGGATGCTAA AACATTCAAA GAATTGGGCC GAGCCACAGT
TAACGTAGAA

1651 ATGCATCTGG ACCTGCATGG GATGTTTATA CCACAGAATG
ATTTGGGGGC

1701 TGAGACGGAA TAAAACGCTA TTGATCCGAC TACACAACT
GAGACAACTT

1751 TCTACTGAAC ATGAGTTAAT ATCCCTTTTA CCATTCAAGA
ACAACCATAT

1801 AACGACACAA AATGACTATG TATAATCTCT TAAATAATAG
ATATAATCCT

1851 TTTAAGGCAC AGCGATGAGT TTTACTACAG GTAACGATAT
GCACAACTGG

1901 CATATAACTA TTCCAAAAGA AGAAGAACGA TCAGTGTTTT
AGAAGTGCTA

1951 ATGTTGTACA TAACGGCGGC AGAGGGAACA GGAGAGAAAG
GTAACGGGAA

2001 TATTTAATAG AATATAGATT TCTGAGCAAA TGAAGTGCAG
TATTTATGGT

2051 GTGATGCATG GCATGAGTCA CATAGGTCTG CAGCTCATGT
ATCTTTTAGA

2101 GATCGTTTCA AGATTGCAGC TTGTGATGCA AGTTTTCTCC
AGCCAGAAAA

2151 CCTCATTTTA AACCATCTGC TACTGGTAAT TCATACCAAT
GCATTTTCTT

2201 GGTGCTCGAT TTACACTATA ACCAAAGTTA AGTATTACAT
TCAGGTGCTA

2251 CAACTTTCTA ATTTACAACC GAAACAAACA AGCAAACAGC
ACTTGCTTTG

2301 CTAATAACCC CATGGTGTAT TTTTCCTTTT TATGATGACA
AAACCAAGTA

2351 CATATGGTTT TATGTAGCAT TCAATTATAC TTCAGTGCTA
TTCCATCCTA

2401 ATGTTATAAG CAATTTGTAT TTAAATCAGT TTTTCCTTGAG
AATATCTGAC

2451 ATAACATTTT GTGTAATGAG ATGACTATGT TGTCTAAAGA
TGAACAGGAA

2501 TGTATCTTTT ATTAGTATTG TTAATTGTGT TACTAATACT
ATGCATATGA

2551 ATGAGAGCAA TGTATTTCTA GGAGAACTCA GATATACATT
CAACAATTTC

2601 TGTAGGTGAA AATGCATTTA CTGATGAAAG TTGAATCGTT
AATGAGGGAG

2651 AAAACTGGGT ATCCATCCAT CCAACTATGT TAGGTGTTCA
CCTGGTCTGT

2701 ATGTGACACC ACGCTGTTTG GGTATCTCTC ACTTTCACAT
ACCTGTTCTC

2751 ATGGTTTCTG CTACTCACTG TATTTTGCAG GAGAGAAACA
AAATGAAATC

2801 ACTGTCACCT ACTATCGCCC CATCACATAA GAACAATGGG
GCTTTGGTGA

2851 CTTGTTTCATG ATTACATAAG ATGTTTGCAg CAGAGCAGCA
ATAGAACCAA

2901 CACCATCCAC AGTTCTTGCT TGCTCTGTTA TGA CTCCCTT
TGCTGTCTTT

2951 ATGGTTTGCA TGTATGAAGA ATACACTGCC TAATTCTAAT
GTTAAAAAGT

3001 CACTGGGGTC AGATCTAGAG CTTAAGTAAG CAGTCTGGGG
TTTTCAAATG

3051 TTTATATGTT CCATAAAATG GAAATAAACA CCTCCATAAT
AAAAAAAAAA

3101 AAAAAAAAAA A

1 METIFNRNKE EHPEPIKAEV QGQLPTWLQG VLLRNGPGMH
TIGDTKYNHW

51 FDGLALLHSF TFKNGEVYR SKYLRSDTYN CNIEANRIVV
SEFGTMAYPD

101 PCKNIFAKAF SYLSHTIPEF TDNCLINIMK TGDDYYATSE
TNFIRKIDPQ

151 TLETLDKVDY SKYVAVNLAT SHPHYDSAGN ILNMGTSIVD
KGRTKYVLFK

201 IPSSVPEKEK KKSCFKHLEV VCSIPSRLL QPSYYHSFGI
TENYIVFIEQ

251 PFKLDIVKLA TAYIRGVNWA SCLSFHKEDK TWFHFVDRKT
KKEVSTKFYT

301 DALVLYHHIN AYEEDGHVVF DIVAYRDNSL YDMFYLLKLD
KDFEVNNKLT

351 SIPTCKRFVV PLQYDKDAEV GSNLVKLPTS ATAVKEKDGS
IYCQPEILCE

401 GIELPRVNYD YNGKKYKYVY ATEVQWSPVP TKIAKLVQVT
KEVLHWGEDH

451 CWPSEPIFVP SPDAREEDEG VVLTCVVVSE PNKAPFLLIL
DAKTFKELGR

501 ATVNVEMHLD LHGMFIPQND LGAETE

10 EEHPEPIKAEVQGQLPTWLQGVLLR..NGPGMHTIGDTKYNHWF DGLALL
57
20 EELSSPLTAHV TGRIP L WLTGSLLRCFTGPGLFEVGSEPFYHLFDGQALL
69
58 HSFTFKNGEVYYRSKYLRSDTYNCNIEANRIVVSEFG..TMAYPDPCKNI
105
70 HKFDFKEGHV TYHRRFIRTDAYVRAMTEKRIVITEFGFTTCAFPDPCKNI
119
106 FAKAFSYLSHTIPEFTDNCLINIMKTGDDYYATSETNFIRKIDPQTLETI
155
120 FSRFFSYFRGV..EVDNALVNVYPVGEDYYACTETNFITKINPETLETI
167
156 ..DKVDYSKYVAVN LATSHPHYDSAGN ILNMGTSIVDKGR TKYVLFKIPS
203
168 FTKQVDLCNYVSVNGATAHPHIENDGTVYNIGNCFCGKNFSIAYNIVKIPP
217
204 SVPEKEKKKSCFKHLEVVC SIPSRLLQPSYYHSFGITENYIVFIEQPFK
253
218 LQADKEDPISKFTS.EIVVQFPCSDRFKPSYVHSFGLTPNYIVFVETPVK
266
254 LDIVKLATAY.IRGVNWASCL.SFHKEDK.TWFHFVDRKTKKEVSTK FYT
300
267 INLFKFLSSWSLWGANYMDCFESFTNETMGVWLHIADKKRKKYLNNKYRT
316
301 DALVLYHHINAYEEDGHVVFDIVAYRDN SL...YDMFYLLKKLDKDFE...
344
317 SPFNLFHHINTYEDNGFLIVDLCCWKGF EFVYNYFTLYLANLRENWEEVK
366
345 VNNKLTSIPTCKRFV VPLQYDKDAEVGSNLVKLP.TSATAV..KEKDGS I
391
367 KNARKAPQPEVRRYVLPLNIDK.ADTGKNLVTLPNTTATAILCSDEFTTI
415
392 YCQPEILCEG....IELPRVNYD.YNGKKYKYVYATEVQWSPVPTKIAKL
436
416 WLEPEVLFSGPRQAFEFPPQINYQKYCGKPYTYAYGLGLNHF.VPDR LCKL
464

437 NVQTKEV LH..WGEDHCWPSEPIFVPSPDAREEDEGVVLT CVVVSEPNKA
484 | | . | | | | | | : | | | | | | | | | | : | | | | . | |
465 NVKTKETWFTVWQEPDSYPSEPIFVSHPDAL EEDDGVVLSVVVSPGAGQK
514
485 P.FLLILDAKTFKELGRA..TVNVEMHLDLHG MF 515
| : | | | | . | | | | . | | : . . | | : |
515 PAYLLILNAKDLSEVARAEFTVEINIPVTFHGLF 548

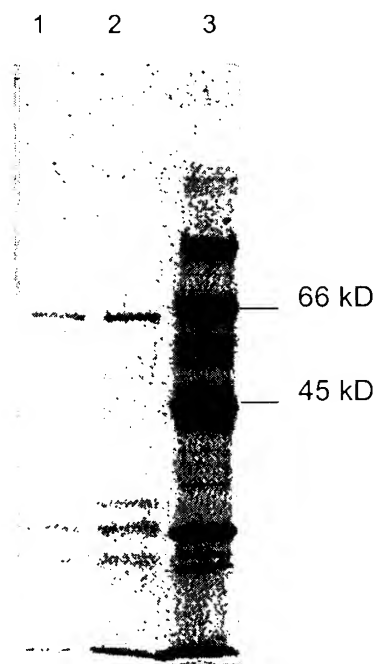


Fig. 6 shows a 10% polyacrylamide gel with E.coli expressed β,β -carotene 15,15'-monooxygenase after affinity tag purification; lane 1 and lane 2: 2 fractions from the Co^{2+} -chelate column showing the main band at 60 kD; lane 3: low range molecular weight marker (Bio Rad).

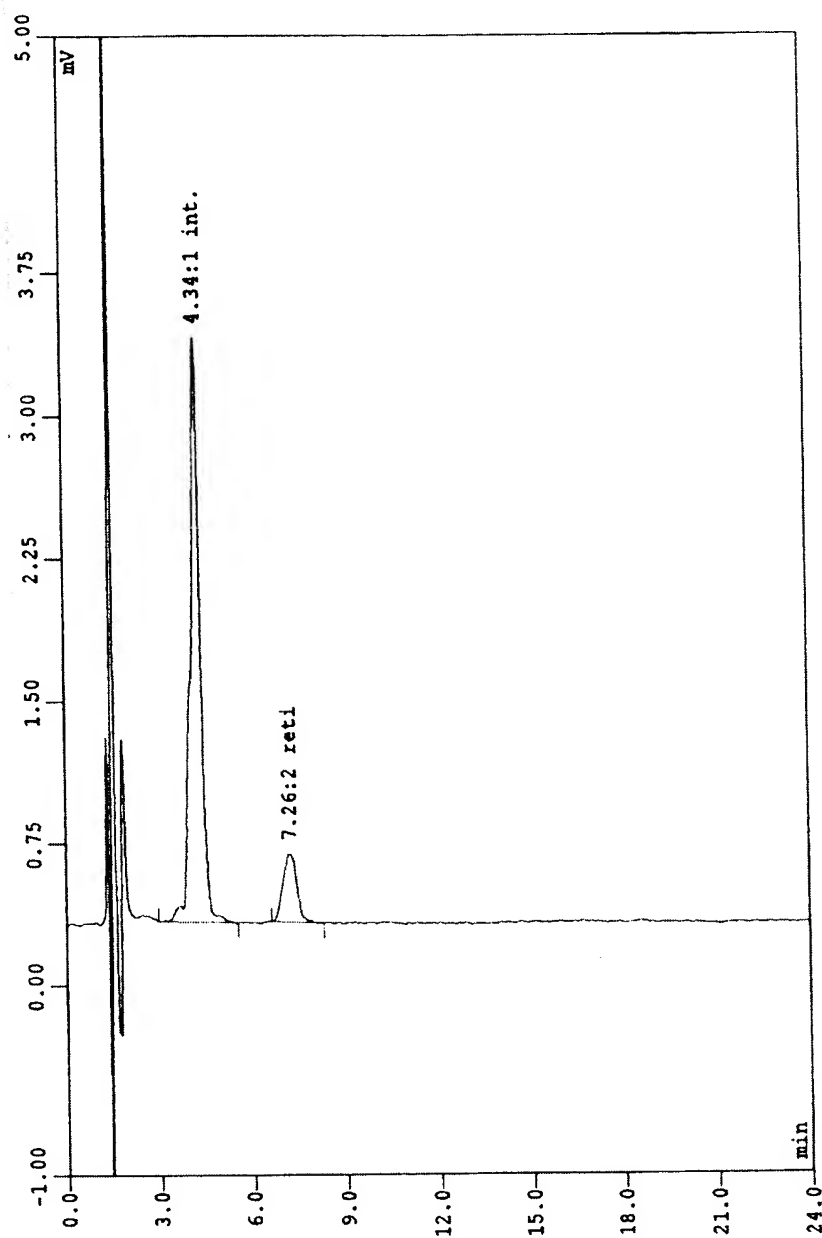


Fig. 7 shows an HPLC profile of the reaction mixture at the end of an activity assay for the β,β -carotene 15,15'-monooxygenase following the procedure in example 1. The first peak in the chromatogram represents the internal standard, while the second peak corresponds to retinal as the only product formed during the central cleavage with β -carotene as substrate.

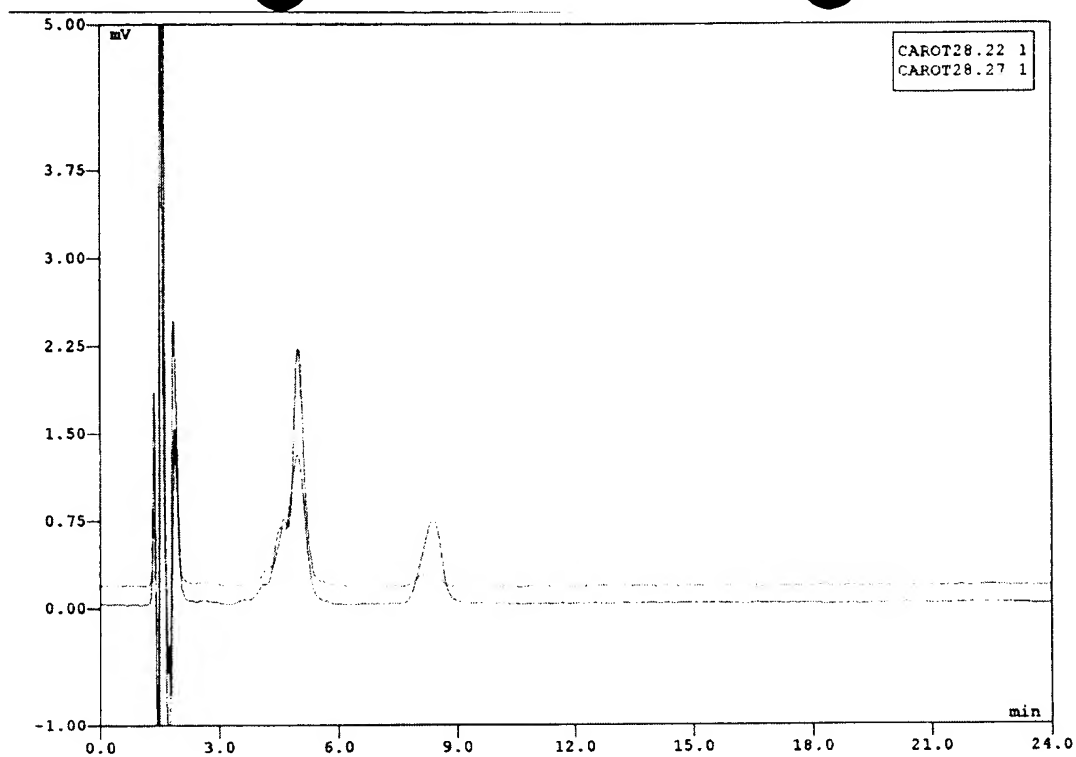


Fig. 8 confirms that the product peak in Fig. 7 is indeed retinal. A sample which was positive in the activity assay (green (upper) chromatogram) was spiked with retinal and analysed in second HPLC run (red (lower) chromatogram). The chromatograms of the two runs were then overlayed.